#10
9-13-01

ATTORNEY DOCKET NO. 13172.0001U1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
Dean *et al.*)
)
Serial No. 09/514,113)
)
Filed: February 28, 2000)
)
For: METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION)

Group Art Unit: 1655

Examiner: Sisson, B.

RESPONSE TO OFFICE ACTION

Assistant Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

September 4, 2001

Sir:

This is responsive to the Office Action dated March 2, 2001. Please amend the above-identified application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 7, line 9, with the following rewritten paragraph:

#1

-- Examples of such nucleotides include abasic nucleosides (Beigelman *et al.*, *Bioorganic & Medicinal Chemistry Letters* 4(14):1715-1720 (1994); Moran *et al.*, *Nucleic Acids Res.* 24(11):2044-

2052 (1996); Matray and Kool, *Nature* 399:704-708 (1999)), 5'-fluoro substituted nucleosides (Robins and Wnuk, *Tetrahedron Lett.* 29:5729 (1988)), 5'-alkyl substituted nucleosides (Ray and Jaxa-Chamiec, *Heterocycles* 31(10):1777-1780 (1990); Jun-Dong and Li-He, *Synthesis* 909-911 (1990); Tanaka *et al.*, *Tetrahedron Lett.* 30:2567-2570 (1989)), nucleosides with 5'-alkyl or phenyl substituted ethers (Jones *et al.*, *Carbohydrates, Nucleosides, Nucleotides* 4:301 (1977)), 5'-substituted thioethers (Connolly and Rider, *Nucleic Acids Res.* 13:4485 (1985); Connolly, *Nucleic Acids Res.* 15:3131-3139 (1987); Sinha and Cook, *Nucleic Acids Res.* 16:2659 (1988); Kumar *et al.*, *Nucleic Acids Res.* 19:4561 (1991); Zuckermann *et al.*, *Nucleic Acids Res.* 15:5305 (1987); Gupta *et al.*, Tetrahedron Lett. 31:2471-2474 (1990); Asseline *et al.*, *Tetrahedron* 48:1233-1254 (1992)), 5'-amines and substituted amines (Connolly and Rider, *Nucleic Acids Res.* 13:4485 (1985); Haralambidis *et al.*, *Nucleic Acids Res.* 15:4857 (1987); Zuckermann *et al.*, *Nucleic Acids Res.* 15:5305 (1987), Li *et al.*, *Nucleic Acids Res.* 15:5275 (1987); Dreyer and Dervan, *Proc. Natl. Acad. Sci. USA* 82:968 (1985)), phosphate esters as 5'-terminators (Tanaka *et al.*, *Tetrahedron Lett.* 30:2567-2570 (1989)), inverted bases or α-nucleosides as 5'-terminators (Bloch *et al.*, *Gene* 72:349 (1988); Sequin, *Helv. Chim. Acta.* 57:68 (1974)), 2',3'-dideoxy nucleosides as 5'-terminators (Huryn and Okabe, *Chem. Rev.* 92:1745-1768 (1992)). --

Please replace the paragraph beginning at page 8, line 1, with the following rewritten paragraph:

-- The nucleotides or oligonucleotides can also be derivatized with, for example, biotin, dyes such as fluorescein or rhodamine, or proteins such as alkaline phosphatase or horseradish peroxidase. 5'-modifications useful in the disclosed oligonucleotides include 5'-spacers (Durand *et al.*, *Nucleic Acids Res.* 18:6353 (1990); Salunkhe *et al.*, *J. Amer. Chem. Soc.* 114:8768-8772 (1992); Dolinnaya *et*

al., *Nucleic Acids. Res.* 21:5403-5407 (1993); Takeshita *et al.*, *J. Biol. Chem.* 262:10171-10179 (1987); Kalnik *et al.*, *Biochemistry* 27:924-931 (1998)), 5'-biotinylated primers (Cocuzza, *Tetrahedron Lett.* 30:6287-6290 (1989); Nelson *et al.*, *Nucleic Acids Res.* 20:6253-6259 (1992)), 5'-cholesteryl (Mackellar *et al.*, *Nucleic Acids. Res.* 20:3411-3417 (1992); Stein *et al.*, Biochemistry 30:2439-2444 (1991)), 5'-DNP-TEG (Will *et al.*, *Carbohydrate Research* 216:315-322 (1991); Grzybowski *et al.*, *Nucleic Acids Res.* 21:1705-1712 (1993)), 5'-psoralen cross-linkers (Pieles and Englisch, *Nucleic Acids Res.* 17:285 (1989); Takasugi *et al.*, *Proc. Natl. Acad. Sci. USA* 88:5602-5606 (1991)), 5'-intercalating agents (Thoung and Chassignol, *Tetrahedron Lett.* 29:5905 (1988)), 5'-PNA conjugates (Nielsen *et al.*, *Science* 254:1497-1500 (1991); Egholm *et al.*, *J. Am. Chem. Soc.* 114:1895-1897 (1992)), 5'-enzyme conjugates (Jablonski *et al.*, *Nucleic Acids. Res.* 14:6115-6128 (1986)), 5'-dye-label (Molecular Probes, Eugene, Oreg.; Research Organics, Cleveland, Ohio). --

A2

Please replace the paragraph beginning at page 21, line 6, with the following rewritten paragraph:

A3

-- Ten reactions were carried out under the conditions used for ERCA in order to illustrate the reduction of primer-based artifacts by using primers containing two template-deficient nucleotides at the 5' ends. Reactions (30 µl) contained 20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM MgSO4, 0.1% TRITON X-100 (pH 8.8 at 25°C) (TRITON is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc., Danbury, Conn.). In addition, reactions contained 400 µM deoxyribonucleoside triphosphates, α-[^{32}P] dCTP, specific activity 169 cpm/pmol total dNTP, and 8 units Bst DNA polymerase. ERCA primers were added as indicated, where 'aba' indicates the presence of an abasic nucleotide residue. --

IN THE CLAIMS

Please amend the claims as follows:

5. (Amended) The method of claim 1 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

6. (Amended) The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

REMARKS

Claims 1-76 are pending in this application. Claims 50-76 have been withdrawn from consideration as being drawn to a non-elected invention. Claims 1-49 are under examination. Claims 5 and 6 have been amended to correctly refer to the "method of claim 1" rather than the "method oligonucleotide of claim 1". Three paragraphs in the specification have been amended. The paragraphs on pages 7 and 8 have been amended to correct reference to the authors of some of the cited publications. The paragraph on page 21 has been amended to identify a trademark that is recited as suggested by the Examiner. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made." No new matter is believed added. Support for these amendments,

if needed, can be found in the specification, and in particular the original claims and relevant paragraphs. In light of the following remarks, Applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

I. Rejection under 35 U.S.C. § 112, first paragraph

Claims 1-49 were rejected under 35 U.S.C. § 112, first paragraph, as the specification allegedly does not reasonably enable methods of amplification besides rolling circle amplification nor the use of primers comprising modified nucleotides other than abasic nucleotides. The Office Action outlines specific factors to be considered, as have been summarized in *In re Wands*, in determining whether a particular disclosure would require undue experimentation. But, at no point, does the Office Action go beyond reciting these factors, as they are perceived by the Examiner, to establish a *prima facie* case that the claimed invention is not enabled by the specification.

Applicants submit that the specification as written does not require undue experimentation and, while not required due to the Office Action's failure to establish a *prima facie* case of nonenablement, Applicants offer the following point-by-point response to each *Wands* factor for the Examiner's consideration.

In response to Examiner's assertion that "the quantity of experimentation need is great, on the order of several man-years and then with little, if any, reasonable expectation of success," Applicants submit that the Examiner has both overestimated the quantity of work required to reduce the claimed invention to practice and has underestimated the level of teaching provided by the specification,

including material incorporated therein by reference. In the case of the present invention, the breadth of the invention necessarily requires some quantity of experimentation. This experimentation, while perhaps greater than is required for inventions with fewer aspects, is not undue. As was summarized in *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (MPEP 2164.06), "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." The guidance provided in the specification fully enables the invention and, as such, consititutes sufficient direction or guidance to the skilled artisan. Applicants submit, therefore, that the quantity of experimentation does not support a finding that the invention is not enabled by the specification.

In response to the Examiner's assertion that the amount of guidance provided is limited to only a small portion of the claimed invention, Applicants submit that while the sections of the specification titled "Examples" describe the use of abasic nucleotides in a primer sequence that is used in rolling circle amplification, the specification provides adequate guidance for the totality of the claimed invention. Specifically, in response to the Office Action's assertion that the provided list of modified or derivatized nucleotides does not enable the incorporation of these into a primer sequence, Applicants submit first that those of skill in the art of oligonucleotide synthesis would know how to incorporate a wide variety of nucleotides in oligonucleotides, and second, that the references cited in the description of nucleotides to be used in the invention (page 7, line 9 to page 8, line 19) include description enabling the synthesis of oligonucleotides containing the modified nucleotides (copies of all these publications were submitted with the Information Disclosure Statement filed June 21, 2000).

In response to the assertion that there is not adequate guidance to the use of the primers, Applicants submit that template-directed primer extension reactions are well-understood to those of skill in the art and follow predictable rules of behavior. As is known to those of skill in the art, the typical template-directed primer extension reaction requires the hybridization of the primer to its template via hydrogen bonding and the extension of the primer by the incorporation of nucleotides or nucleotide analogs by the polymerase present. The hybridization of a primer to a template sequence is exceptionally straightforward (see U.S. Patent 6,027,923, column 11, lines 10-32 and line 56 to column 12, line 3). Also, as it too reflects the same straightforward phenomenon, the binding of the primers to the truncated, less than full length product strands is also straightforward. As would also be recognized by those of skill in the art, the specific conditions and requirements for each of the different methods of amplification will vary depending upon the specific method as will the suitable template-deficient oligonucleotide. Determination and use of such conditions is well within the skill of those in the art. Furthermore, the specification provides adequate description for the different amplification methods as the cited references on page 9, line 13 to page 10, line 5, describe all aspects of these methods as is necessary to practice them, including guidance as to the use of primers.

Importantly, it is well-known that the practice of any of these amplification methods requires a measure of empirical study and evaluation to determine the appropriate sequences and conditions for the optimal performance. The levels of study and evaluation for the present invention are not significantly greater, nor more complicated, than that for the practice of any of the standard, referenced amplification methods. Specifically, the optimization of primer composition and/or sequence and the use of primers is within the skill of one in the art given the teaching provided in the specification as to

the required properties of the primers, see for example, page 8, lines 20-26 and page 12, lines 20-26. Applicants submit that consideration of the amount of direction or guidance provided does not support a finding that the invention is not enabled by the specification.

In response to Examiner's intimation that the working examples, limited to the use of abasic nucleotides in primer sequences subsequently used in rolling circle amplification, are insufficient to support enablement of the claimed genus, Applicants submit that the working examples and the specification as a whole are more than adequate to provide many enabled aspects of the current invention. Further, the Applicants submit that the situation at hand is not analogous to that in *Genentech v. Novo Nordisk A/S*, Slip Op. No. 96-1440 (Fed. Cir. March 13, 1997), wherein the Federal Circuit invalidated Genentech's patent solely on the ground that the specification failed to provide an enabling disclosure of the claimed invention. In *Genentech*, the single claim was directed to a method of making an hGH product via a cleavable fusion method. In support of this method, as described by the Court, "... no reaction conditions for the steps needed to produce hGH are provided; no description of any specific cleavable conjugate protein appears. The relevant portion of the specification merely describes three (or perhaps four) applications for which cleavable fusion expression is generally well-suited and then names an enzyme that might be used as a cleavage agent (trypsin), along with sites at which it cleaves ("arg-arg or lys-lys, etc."). Thus, the specification does not describe a specific material to be cleaved or any reaction conditions under which cleavable fusion expression would work."

This is in stark contrast to the present application which provides, in addition to the two working examples, specific examples of template-deficient nucleotides and oligonucleotides, description of how

to make template-deficient oligonucleotides for use as primers, specific nucleic acid amplification reactions which can be used, description of reaction conditions to be used (incorporated by reference), and reference to optimization of conditions. Consequently, Applicants submit that the present application does not merely offer "...vague intimations of general ideas that may or may not be workable. . .," but instead provides the reasonable detail, including the novel aspects of the invention, that is required to enable an invention. Applicants submit that consideration of the presence or absence of additional working examples does not support a finding that the invention is not enabled by the specification.

In response to the Examiner's statement that the claimed invention relates directly to matters which are inherently unpredictable and as such, require greater levels of enablement, Applicants submit that the art most closely related to the present invention is predictable. Specifically, the design and use of primers in nucleic acid amplification procedures is well-understood. For example, Wallace, column 11, lines 10-33 and lines 56-62, illustrates the high level of predictability in both the design of oligonucleotide primers and in the optimization of temperature cycling conditions for the use of any given set of primers. This knowledge, coupled with the disclosed techniques of nucleic acid amplification and the well-characterized properties of the different polymerases utilized, provide a high level of predictability. As it is noted in Wallace, column 11, lines 56-62, "[t]he design of primers that bind at preselected temperatures is within the skill of molecular biologists. The temperature at which a specific primer will function can be predicted by available algorithms... ...and by computer programs... based upon primer length and base composition." As such, Applicants submit that the design and use of primers rises to the level of being predictable and that their performance characteristics can be

predicted by resort to known scientific laws. Further, primer design and use can be optimized using techniques and principles known to those of skill in the art.

Thus, Applicants submit that the higher standards for what is required to provide enablement (those standards drawn from *In re Fisher*, cited by the Examiner) are not applicable to the present invention. *In re Fisher* states that "most chemical reactions and physiological activity" involve unpredictable factors and, for cases involving unpredictable factors, a greater level of description were required to provide enablement. It does not state that all cases involving chemical reactions or physiological activity involve unpredictable factors. It only states that most do. The Court recognized that some cases involving chemical reactions or physiological activity do instead involve "predictable factors." Further, *In re Fisher* characterizes cases involving predictable factors as those where "other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws." Based on those same criteria, and the evidence presented above indicating that different embodiments can be made easily and with great predictability as regards their characteristics, Applicants submit that the current invention falls into the case described specifically in *In re Fisher* as not requiring the greater level of description often required of biotechnological inventions to provide enablement. However, even if a greater level of description were required, this level is fulfilled by the specification as outlined above. Therefore, Applicants submit that the nature of the present invention supports a finding that the specification enables the claimed method.

Applicants welcome the Examiner's acknowledgement of the state of the prior art as being severely limited in respect to the use of template-deficient oligonucleotides. Further, Applicants submit that the use of template-deficient oligonucleotides as claimed is entirely unknown. However, such

limitations are not implicated in a reasonable enablement analysis of the claimed method. Rather, the claimed method requires only the application of established skills in a new context. In respect to the synthesis of oligonucleotides which can be used as template deficient oligonucleotides and the nucleic acid amplification reactions in which the template-deficient oligonucleotides can be used, Applicants freely admit that both fields are exceptionally well-developed. Consequently, Applicants submit that the state of the prior art supports a finding that the specification enables the claimed method.

Applicants acknowledge the Examiner's assertion that the relative skill of those in the art is high, on par with those that hold a Ph.D. in biochemistry. While not admitting that the asserted level of skill is correct, Applicants submit that, if true, such a level of skill in the art would necessarily accommodate a relatively high level of experimentation and problem-solving. Furthermore, Applicants submit that the level of experimentation required does not rise to an undue level of experimentation for even those of much lesser skill.

Applicants acknowledge Examiner's statement in regard to the great breadth of the claims, but assert that, for reasons discussed above, this does not indicate that the claimed invention lacks enablement.

In summary, Applicants submit that the *Wands* factors support a finding of enablement for the claims. For this and all of the above reasons, Applicants submit that claims 1-49 are fully enabled.

II. Rejection under 35 U.S.C. § 102

A. Claims 1-2, 5, 11, 20-21, 23, 28, 32, 36, 39, 41, 42-44, 46 and 49 are rejected under 35 U.S.C. § 102 (a) as allegedly being anticipated by Wallace. Specifically, the disclosure of Wallace, column 9, 3rd and 4th paragraphs, discloses the use of abasic nucleotides in a primer used in an amplification reaction and that the primers are alleged to contain a plurality of "non-replicable and/or cleavable elements." Further, Wallace allegedly teaches that the modified nucleotide is not at the terminal residue of any of the primers.

Applicants respectfully disagree that Wallace anticipates any of the claims. Claim 1 of the present application specifically recites that the "...number and composition of template-capable nucleotides 3' of the template deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction." Correspondingly, one of the properties of any primer of claim 1 is that the sequence of the template-deficient oligonucleotide 3' of the template-deficient oligonucleotide is sufficient both in terms of the composition and the number of nucleotides to hybridize effectively to the sequence of the template strand provided and to effectively prime synthesis. However, the primers of Wallace are specifically designed to be, and are required to be, incapable of effectively priming nucleic acid synthesis in the nucleic acid amplification reaction when only the sequence 3' to the non-replicable element is presented with complementary sequence. This follows from the goal of Wallace to prevent replication of second generation primer extension products. Specifically, in column 2, lines 49 - 53, in reference to the reactions wherein the primers are used, Wallace recites "...wherein the second generation primer

extension products contain at least a portion of the nucleic acid sequence of interest and cannot serve as templates for the synthesis of extension products of the primers which were extended to synthesize their templates." In other words, claim 1 of the present application requires that the template-deficient primer and the extended products of that primer be capable of promoting extension of the primer and Wallace requires that his primer and the extended products of that primer, specifically the second-generation products, be incapable of promoting extension of the primer. Thus, the primers of Wallace, and thus, the method of Wallace, do not anticipate the current invention. Applicants respectfully request removal of this ground of rejection.

B. Claims 1, 3, 5, 11, 13-16, 19-20, 23-25, 28-32, 34, 36-37, 41-43 and 46-49 are rejected under 35 U.S.C. § 102(3) as allegedly being anticipated by Todd et al. Specifically, the disclosure of Todd et al., column 11, Example 3, is alleged to anticipate the above-indicated claims as it discloses the use of chimeric primers in a PCR reaction and teaches that "one or two" ribonucleotides be present in the primers.

Applicants' respectfully disagree. Claim 1 of the present application specifically recites that the "...number and composition of template-capable nucleotides 3' of the template deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction." In the case of the cited chimeric primer of Todd et al., whose sequence is given at column 11, line 64, the ribonucleotides are incorporated at the 2nd and 3rd position from the 3' end of the primer. Thus, the chimeric primer of Todd et al. provides only a single template-

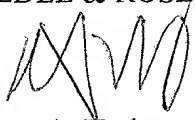
capable nucleotide residue 3' of the template-deficient nucleotide closest to the 3' end of the oligonucleotide. This, Applicants submit, is an insufficient number of nucleotides to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction. Therefore, Todd et al. does not anticipate claim 1, nor does it anticipate any claim dependent therefrom. Applicants respectfully request removal of this ground of rejection.

Pursuant to the above remarks, consideration and allowance of the pending application is believed warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A check in the amount of $445.00 is enclosed for a 3 month extension of time (small entity). The amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 14-062.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.



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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date shown below.

Robert A. Hodges

9/4/01
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